d.) Remarks

Any inquiry concerning this communication or earlier communications from the applicant should be directed to Chuan Li whose telephone number is (858) 361-7231. The applicant can normally be reached from 9:00 a.m. to 5:00 p.m. pacific standard time.

The applicant may also be reached at Expression Technologies Inc. at (858) 558-1861 or by fax at (858) 558-1883 or by email at chuanli@exptec.com.

Applicant Name: Chuan Li

Signature: 

Date: December 17, 2009

Sequence ID NO: 1

BIO•SYNTHESIS

Lot No: B716-1

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 1** 1C2C0131
Primer Lot Number:	**B716-1**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CGC CCG CCG CCC GGG CGC CCC GCC TTC CGC TTC CTC GCT CAC TG**

Primer Data

Primer Length:			44		
Type:			DNA		
Composition:	A	C	G	T	Others
	1	25	11	7	0
	2.3%	56.8%	25.0%	15.9%	0.0%

Molecular Weight (Ammonium Salt):	13231.8
Exact Weight per OD (Ammonium Salt):	37.87
Nanomoles per OD (Ammonium Salt):	2.86
Micromolar Extinction Coefficient:	349.38
Total ODs in This Tube:	5
Total Amount in ug:	189.36
Total Amount in nmoles:	14.31
Purification:	Desalted
Melting Temperature in Celsius:	160.0

5' END	OH
3' END	OH

Note: OD WILL VARY

biosyn@biosyn.com

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Sequence ID NO: 24

BIO•SYNTHESIS

Lot No: B716-3

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 3** IC2C0133
Primer Lot Number:	**B716-3**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CGC CCG CCG CCC GGG CGC CCC GCC AAC GCG GAA GTC AGC GCC CT**

Primer Data

Primer Length:			44		
Type:			DNA		
Composition:	A	C	G	T	Others
	5	23	14	2	0
	11.4%	52.3%	31.8%	4.5%	0.0%

Molecular Weight (Ammonium Salt):	13372.8
Exact Weight per OD (Ammonium Salt):	34.85
Nanomoles per OD (Ammonium Salt):	2.61
Micromolar Extinction Coefficient:	383.67
Total ODs in This Tube:	5
Total Amount in ug:	174.27
Total Amount in nmoles:	13.03
Purification:	Desalted
Melting Temperature in Celsius:	162.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 3

BIO•SYNTHESIS

Lot No: B716-4

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 4** 10,20 0331
Primer Lot Number:	**B716-4**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CGC CCG CCG CCC GGG CGC CCC GCC AAC GCA GAC CGT TCC GTG GC**

Primer Data

Primer Length:			44		
Type:			DNA		
Composition:	A	C	G	T	Others
	4	23	14	3	0
	9.1%	52.3%	31.8%	6.8%	0.0%

Molecular Weight (Ammonium Salt):	13363.8
Exact Weight per OD (Ammonium Salt):	35.38
Nanomoles per OD (Ammonium Salt):	2.65
Micromolar Extinction Coefficient:	377.73
Total ODs in This Tube:	5
Total Amount in ug:	176.9
Total Amount in nmoles:	13.24
Purification:	Desalted
Melting Temperature in Celsius:	162.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 4

BIO•SYNTHESIS

Lot No: B716-5

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 5** 3C015
Primer Lot Number:	**B716-5**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**CCG CCG CGC CGC TTC CAC TGA GCG TCA GAC CC**

Primer Data

Primer Length:	32
Type:	DNA

Composition:

A	C	G	T	Others
4	16	8	4	0
12.5%	50.0%	25.0%	12.5%	0.0%

Molecular Weight (Ammonium Salt):	9668.4
Exact Weight per OD (Ammonium Salt):	34.97
Nanomoles per OD (Ammonium Salt):	3.62
Micromolar Extinction Coefficient:	276.48
Total ODs in This Tube:	5
Total Amount in ug:	174.85
Total Amount in nmoles:	18.08
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 5

BIO•SYNTHESIS

Lot No: B716-7

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 7** IGAN
Primer Lot Number:	**B716-7**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG TTC GGG GAA ATG TGC GCG GA**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	4	6	18	4	0
	12.5%	18.8%	56.3%	12.5%	0.0%

Molecular Weight (Ammonium Salt):	10068.4
Exact Weight per OD (Ammonium Salt):	31.85
Nanomoles per OD (Ammonium Salt):	3.16
Micromolar Extinction Coefficient:	316.08
Total ODs in This Tube:	5
Total Amount in ug:	159.27
Total Amount in nmoles:	15.82
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 6

BIO•SYNTHESIS

Lot No: B716-8

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 8** IGKN
Primer Lot Number:	**B716-8**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG TTG TCG GGA AGA TGC GTG AT**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	4	5	17	6	0
	12.5%	15.6%	53.1%	18.8%	0.0%

Molecular Weight (Ammonium Salt):	10058.4
Exact Weight per OD (Ammonium Salt):	31.95
Nanomoles per OD (Ammonium Salt):	3.18
Micromolar Extinction Coefficient:	314.82
Total ODs in This Tube:	5
Total Amount in ug:	159.75
Total Amount in nmoles:	15.88
Purification:	Desalted
Melting Temperature in Celsius:	108.0

5' END	OH
3' END	OH

Note: OD WILL VARY

Sequence ID NO: 7

BIO•SYNTHESIS

Lot No: B716-9

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 9** IGTN'
Primer Lot Number:	**B716-9**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG TTC TCA TGT TTG ACA GCT TA**

Primer Data

Primer Length:	32
Type:	DNA

Composition:

A	C	G	T	Others
4	7	12	9	0
12.5%	21.9%	37.5%	28.1%	0.0%

Molecular Weight (Ammonium Salt):	9903.4
Exact Weight per OD (Ammonium Salt):	33.11
Nanomoles per OD (Ammonium Salt):	3.34
Micromolar Extinction Coefficient:	299.07
Total ODs in This Tube:	5
Total Amount in ug:	165.57
Total Amount in nmoles:	16.72
Purification:	Desalted
Melting Temperature in Celsius:	102.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 8

BIO•SYNTHESIS

Lot No: B716-10

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 10** IGCN
Primer Lot Number:	**B716-10**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GGG CGG CGG GCG AAG CCA CTG GAG CAC CTC AA**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	7	10	13	2	0
	21.9%	31.3%	40.6%	6.3%	0.0%

Molecular Weight (Ammonium Salt):	9910.4
Exact Weight per OD (Ammonium Salt):	31.42
Nanomoles per OD (Ammonium Salt):	3.17
Micromolar Extinction Coefficient:	315.45
Total ODs in This Tube:	5
Total Amount in ug:	157.08
Total Amount in nmoles:	15.85
Purification:	Desalted
Melting Temperature in Celsius:	110.0

5' END	OH
3' END	OH

Note: OD WILL VARY

Sequence ID NO: 9

BIO•SYNTHESIS

Lot No: B716-11

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 11** 3GAC
Primer Lot Number:	**B716-11**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG TAC GGG GTC TGA CGC TCA GT**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	3	9	15	5	0
	9.4%	28.1%	46.9%	15.6%	0.0%

Molecular Weight (Ammonium Salt):	9939.4
Exact Weight per OD (Ammonium Salt):	33.32
Nanomoles per OD (Ammonium Salt):	3.35
Micromolar Extinction Coefficient:	298.26
Total ODs in This Tube:	5
Total Amount in ug:	166.62
Total Amount in nmoles:	16.76
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

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Sequence ID NO: 10

BIO•SYNTHESIS

Lot No: B716-12

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 12** 3GKC
Primer Lot Number:	**B716-12**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG ATC GCC CCA TCA TCC AGC CA**

Primer Data

Primer Length:			32		
Type:			DNA		
Composition:	A	C	G	T	Others
	5	14	10	3	0
	15.6%	43.8%	31.3%	9.4%	0.0%

Molecular Weight (Ammonium Salt):	9757.4
Exact Weight per OD (Ammonium Salt):	33.61
Nanomoles per OD (Ammonium Salt):	3.44
Micromolar Extinction Coefficient:	290.34
Total ODs in This Tube:	5
Total Amount in ug:	168.03
Total Amount in nmoles:	17.22
Purification:	Desalted
Melting Temperature in Celsius:	112.0

5' END	OH
3' END	OH

Note: OD WILL VARY

Sequence ID NO: 11

BIO•SYNTHESIS

Lot No: B716-13

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 13** 3GTC
Primer Lot Number:	**B716-13**
Author:	**MD**
Synthesis Scale:	**50 nmole**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG TTC ACG TTC GCT CGC GTA TC**

Primer Data

Primer Length:	32
Type:	DNA

Composition:

A	C	G	T	Others
2	11	12	7	0
6.3%	34.4%	37.5%	21.9%	0.0%

Molecular Weight (Ammonium Salt):	9825.4
Exact Weight per OD (Ammonium Salt):	34.87
Nanomoles per OD (Ammonium Salt):	3.55
Micromolar Extinction Coefficient:	281.79
Total ODs in This Tube:	5
Total Amount in ug:	174.34
Total Amount in nmoles:	17.74
Purification:	Desalted
Melting Temperature in Celsius:	110.0

5' END	OH
3' END	OH

Note: OD WILL VARY

Sequence ID NO: 12

BIO•SYNTHESIS

Lot No: B716-14

Oligo Data Sheet

Date Created:	**3/11/98**
Your Reference ID:	**OLIGO 14** 3GCC
Primer Lot Number:	**B716-14**
Author:	**AH**
Synthesis Scale:	**50 nmol**
Primer Sequence (5' to 3'):	**GCG GCG CGG CGG AAG CAC ACG GTC ACA CTG CT**

Primer Data

Primer Length:	32
Type:	DNA

Composition:

A	C	G	T	Others
6	11	12	3	0
18.8%	34.4%	37.5%	9.4%	0.0%

Molecular Weight (Ammonium Salt):	9861.4
Exact Weight per OD (Ammonium Salt):	32.27
Nanomoles per OD (Ammonium Salt):	3.27
Millimolar Extinction Coefficient:	305.55
Total ODs in This Tube:	5
Total Amount in ug:	161.37
Total Amount in nmoles:	16.36
Purification:	Desalted
Melting Temperature in Celsius:	110.0

5' END	OH
3' END	OH

Note: OD WILL VARY

↓ 14K 10'

↓ 1ml 70% EtOH

↓ 2'

↓ air dry

pellets

The Amount of DNA of #2 and #4 products is about 18ug. amount of DNA of #7, #8, #9 and #4 products is about 28 ug. 90ul and 140ul H2O to make their final concentration at about 0.2 u/ul. p them at −20°C.

4 PCR OS 6-19-98

Now I am doing PCR analysis since the PCR Test worked fine.

DNA	Primers	Product	bps	Comments
pBR322, PstI	3+5	01	1535	
"	1+5	02	813	
pUC19, RI	1+5	03	813	
pACYC177, BHI	4+5	04	1011	
"	1+5	05	726	
pBR322, PstI	2+5	06	1057	
pACYC177, BHI	1+6	07	699	
"	4+6	08	979	
pACYC184, BHI	1+6	09	694	
pBR322, PvuII	7+11	S1	1130	
pUC19, RI	7+11	S2	1130	
pACYC177, BHI	7+11	S3	1130	
"	8+12	S4	1219	
pBR322, PvuII	9+13	S5	1552	
pACYC184, BHI	10+14	S6	1104	

DNA are from page 6. They are diluted to 1 ng/ul and 1 ul was 1 for each reaction.

Primers are from page 05. The estimated concentration is about 8/ul. 2 ul of each primer was used.

Dilute the DNA into appropriate concentration.

Making master mix as on page 6 except the number of reactions ld be 16 instead of 12. One extra is for negative

To Page No. 9

sed & Understood by me,	Date	Invented by	Date
	6/18/98		6-19-98
		Recorded by	

Page No. 8 control.

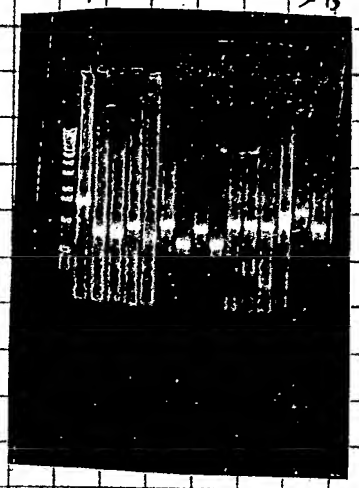
The PCR condition is same as on page 7.

100 ul of each PCR product

↓ take out 3ul

6/24/98 ↓ run on 0.8% agarose TBE

← ↓ take a picture



All the reactions worked. However, some worked better than others. #1 to 5 and 10 to 14 seems have many non-specific products. By increasing the template concentration, specific product may be increased and non-specific products may be decreased.

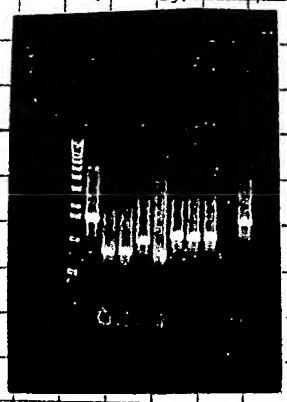
6-20-98 → Repeat reactions #1 to 5 and 10 to 14 by increasing the template 10X (use 10ng)

100 ul of each PCR product

↓ take out 3ul

↓ ran on 0.8% agarose TBE

6/24/98 ← ↓ take a picture



All the reactions appear to be better except #13. I probably make mistake when adding template or primers for #13.

Keep the PCR products at -20°C

5. PCR SI

6-21-98

DNA	Amount	Primers	P	bps	Comments
pACYC177 BH1	1 ng	8 + 12	S4	1219	
"	10 ng	"	"	"	
pACYC177 PstI	1 ng	"	"	"	
"	10 ng	"	"	"	
pEX -3X	1 ng	15 + 17	I	1198	

The primers are from page 5. 2 ul of each primer was used. The PCR condition is same as on page 7.

To Page No. 10

Read & Understood by me,
STEVEN F. GESSERT
Date 6/24/98

Invented by Chuanli
Recorded by Chuanli
Date 6-20-98, 6-21-98

Page No. 92

(2) By comparing the yields of the miniprep's ~~on~~ between page 11 and 92, it is clearer that the copy number of plasmids is determined by the selection marker it contains. In OS4 miniprep, the A based plasmids give very low yields except #15.

(3) ~~The~~ SmaI has two sites on OS4 constructs. Therefore two bands generated after SmaI digestion. However the size of the DNA appear to be different even from the colonies ~~on~~ picked from same plate. This observation may be curtifacts of electrophoresis, but I need pay attention on this observation in further analysis.

22. OS Mediprep Test 1 9.8.98

Use the residual ON cultures from 4 ml innoculation (These residual ones are kept at 4°C). to seed 5 ml LB with appropriate antibiotic. prep number, page and ON culture number are indicated in the following table.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
84	84	~~84~~	87	87	85	87	85	91	~~91~~		91		92		
2	28	1	24	18	22	21	25	19	8	13	16	2	8	10	15
Amp				Tet				Chl				Kan			

The above table is messy. I will re-prepare the table below:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Amp						Tet		Chl				Kan			
84	84	~~84~~	~~84~~	87	87	85	87	85	91		87	91		92	
2	28	16	4	18	22	21	25	19	8		13	16	2	8	10	15

5 ml innoculated LB w/ appropriate antibiotic

↓ 37°C w/ shacking at 300 RPM for 3hs

1 to 4, 5, 7, 13 and 15 grows fast.
6, 8, 9 to 12, 14 and 16 grow slowly.

↓ 37°C for another 3h w/ shacking

9 to 12, 14 and 16 still do not grow well

↓ 37°C w/ shacking o/n. ~13hs.

9.9.98 innoculate all 5 ml into 50 ml LB w/ antibiotic

↓ 37°C for 1h w/ shacking

Test OD590

#1 : 0.435 #12 : 0.278

To Page No. 94

sed & Understood by me,	Date	Invented by	Date
[signature]	9-11-98	[signature] Li	9.8.98
		Recorded by [signature] Li	

↓ 37°C for another 1h w/ shaking.

Test OD575

#1 : 0.718 #12 : 0.283

#8 : 0.676 #16 : 0.257

The final concentration of chl 5×35/50 = 10 ug/ml

↓ add 15 ul 35mg/ml chl to 1-8 and 13-16

↓ 37°C w/ shaking for 4 hs

#1 : 1.260 #12 : 1.192

#8 : 0.992 #16 : 0.337

↓ 3.2K for 15' (actually 10' should be enough)

pellets (#16 has smallest pellet)

also has a small pellet and 11 have biggest pellets, are most viscous after suspend the cells

↓ 0.5 ml LETT

↓ completely resuspend cells by pipetting

↓ transfer to eppendorf tubes

↓ boil for ~~90°~~ → 120°

↓ 14K 10'

after 10' spinning, #1-5, 7&8, 13-15 did not pellet, boil in for another 3' and repeat spinning.

after second spin, they form pellets. Freeze them in dry ice/EtoH bath, thaw them, spin for another 10' (5, 7, 14, 15 are treated dry ice/EtoH bath)

adjust the supernatants to the same volume by LETT

*1 → *2 → *4

↓ transfer supernatants to new tubes

supernatants *3

↓ 1V ϕ:chl ext.

↓ 2V EtoH

↓ 5' 14K

pellets

↓ 70% EtoH Wash

pellets

↓

To Page No. 95

ssed & Understood by me,	Date	Invented by	Date
	3-11-98		9.9.98
		Recorded by	

*4 The lysed cell pellets are different in size: The approximate pellet size (CAPS) are listed in the following table:

up#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PS	400	400	300	250	400	100	250	200	200	200	150	120	100	250	250

The approximate pellet sizes (CAPS) are in microliters.

pellets after 70% EtoH wash are different in size. Their relative size are listed in the following table:

up#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ze	L	L	L	L	L	~~M~~S	L	M	S	S	S	VS	L	L	L

↓ air dry the pellets O/N.

↓ re-suspend in 200ul TE w/RNase A 9.10

After O/N air dry, the pellets are difficult to be dissolved especially the larger pellets. When they are finally dissolved (take about 2hs w/ vortexing), they form heavy foams while vortexing.

Add another 200ul TE w/RNase A to large pellet tubes namely #1-5, 7, 13-15.

It is amazing that all the pellets seem dissolve completely these efforts.

SmaI digestion:

Master Mix for each RXN		16 RXNs
H_2O	6.9 ul	110.4
10X Buffer	1 ul	16
SmaI	0.1 ul	1.6
	8 ul/RXN	128 ul totally.

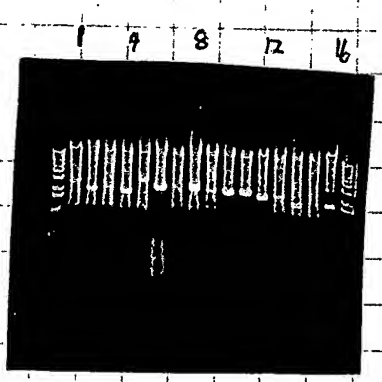
take out 2ul from each sample

↓ add 8ul master mix

↓ R.T for 1h

↓ run on 0.8% agarose TBE

↓ take a picture. (f16, 1/2 sec,



Result analysis:

① Genomic DNA contamination is serio possible solutions ② Decrease the boiling t ⑥ Do not use pepitt to resuspend the cell ⓒ Decrease the Triton-X100 Concentration.

② Most of the preps have enough DNA for future usage. (however 3, 7 and 15 app to have very little DNA (0.025 per band).

possible solutions ④ use higher concentration of chloramphenicol on

To Page No.

tnessed & Understood by me,	Date	Invented by	Date
	9-11-98	Recorded by	9-9-10-98